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Amparoina spinosissima: a continental Asian record and some taxonomic observations

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Abstract —*Amparoina spinosissima* is described and illustrated from Kerala State, India. This is the first record of the species from continental Asia. Basidiospores of the Indian specimens are inamyloid in support of Singer's original observation.

Key words — *Agaricales, Basidiomycota,* floristics, systematics, *Tricholomataceae*

Introduction

The genus *Amparoina* Singer (*Agaricales, Tricholomataceae*), although little known, has a chequered taxonomic history. The type species of the genus, *A. spinosissima*, was originally described as *Marasmius spinosissimus* and was first discovered in Argentina (Singer 1950). Singer (1958) erected *Amparoina* to accommodate *M. spinosissimus,* which he (Singer 1958, 1976) interpreted as having inamyloid spores, an epicutis covered by cherocytes (loose, globose cells with long excrescences or spines; Singer 1986), and a secotioid habit. Later, Singer (1976) proposed a monotypic family, *Amparoinaceae* Singer, and excluded it from *Agaricales.* Singer (1976) also added a second species to *Amparoina, A. heteracantha* Singer. Horak (1980), based on his own collections of *A. spinosissima* made in Argentina and New Caledonia, concluded that the species is not secotioid. After examining the type material of *A. heteracantha*, Horak (1980) considered it to be conspecific with *A. spinosissima.* Horak (1968, 1980), however, never questioned the autonomy of *Amparoina.* Although Singer (1983) did not agree with Horak's merging of the two *Amparoina* species, he conceded that *A. spinosissima* was not secotioid. On the basis of Horak's observations, Singer (1986) reinstated *Amparoina* in the *Tricholomataceae* (*Agaricales*).

Based on the study of several collections of *A. spinosissima* made from Colombia, Puerto Rico, and Hawaii, Desjardin (1995) agreed with most of

Horak's conclusions. However, he found the basidiospores to be amyloid in the specimens he examined and this prompted him to transfer the species to *Mycena* sect. *Sacchariferae*. Although Singer observed the basidiospores of *A. spinosissima* to be inamyloid, this cannot be confirmed as the holotype of *A. spinosissima* no longer exists. Horak's observations (1968, 1980, and his pers. comm. quoted by Desjardin 1995) on the amyloidy of basidiospores from his collections of *A. spinosissima* were not consistent. Takahashi (1999) observed amyloid spores in Japanese collections of the species. We did not re-examine spores from the collections made by Horak and Takahashi, so that the possible variation in amyloid reaction remains an open question. Meanwhile, taxonomic and nomenclatural resources such as the Dictionary of the Fungi (Kirk et al. 2008) and the Index Fungorum (www. indexfungorum.org) continue to recognize *Amparoina*. We accept this point of view for the time being and note that molecular analyses may clarify the relationships among *Amparoina*, *Mycena,* and other agarics in the future. *Mycena* in the present wide sense includes also some species with inamyloid spores as well as species with cherocytes, similar to those of *Amparoina,* on the pileus surface but with amyloid spores (Singer 1986).

Although only rarely collected, *A. spinosissima* is known thus far from Argentina, Colombia, Hawaii, Japan, New Caledonia, and Puerto Rico. During our studies on the agarics of Kerala State, India, we collected a decaying twig bearing primordia of this species, which when incubated in the lab yielded well-developed basidiomata. We present here a full description of the Indian collection along with some taxonomic observations.

Materials and methods

Conventional morphology-based methods were employed for this study. Microscopic observations were made on material stained with 1% aqueous solutions of phloxine and Congo red and mounted in 3% aqueous KOH. Melzer's reagent was used to observe whether the spores and tissues were amyloid. For statistical evaluation 40 spores (20 basidiospores each from two specimens) were measured. The examined collection cited is deposited at the Kew (Mycology) Herbarium.

Taxonomy

Amparoina spinosissima (Singer) Singer, Mycologia 50: 110. 1958. FIGURE 1A–E

≡ *Marasmius spinosissimus* Singer, Schweiz. Z. Pilzk. 28: 193. 1950.

≡ *Mycena spinosissima* (Singer) Desjardin, Bibliotheca Mycol. 159: 15. 1995.

BASIDIOMATA small, delicate. PILEUS 2–5.5 mm wide, 2–4.5 mm high, initially conical, becoming broadly campanulate; surface white to whitish all over,

entirely covered in the primordial stage with a universal veil made up of pale greenish or ivory-colored, erect or curved, conic, detersile spines up to 0.75 mm long that disappear first from the middle, then from the margin and finally from the pileus disc with age, pruinose, dry, very thin, translucently striate, becoming slightly plicate towards the margin; margin initially straight and appendiculate with spines, becoming plane and undulate or finely torn with age. Lamellae adnexed, fairly close, 15–20 reaching the stipe, with lamellulae in 1–3 tiers, ventricose, up to 0.5 mm broad, white; edge finely torn under a lens. Stipe 20–38 × 0.5–1.25 mm, central, terete or slightly compressed, almost equal or with a slightly dilated apex, hollow; surface translucent–white, dry, densely pruinose to hirsute towards the base, almost glabrous at apex; base often subbulbous, not discoid. Context very thin. Odor not distinctive.

Basidiospores (6–)8–9.5(–12) × 4.5–6(–9.5) (8.86 ± 0.17 × 5.96 ± 0.15) µm, Q = 1.24–1.73, Qm = 1.5, ellipsoid, ovoid or rarely subamygdaliform, thin-walled, smooth, with refractive guttules, inamyloid. Basidia 11–18 × 6–11.5 µm, broadly clavate to almost subglobose, thin-walled, hyaline, 4-spored; sterigmata up to 4 µm long. Lamella-edge sterile. Cheilocystidia 7–23.5 × 5–12.5 µm, cylindrico-clavate, subglobose or vesiculose, covered entirely or at least at the apex with minute excrescences, occasionally smooth, thin- to slightly thick-walled (0.5 µm), hyaline; excrescences 0.5–0.75 µm long, cylindrical or subconical. Pleurocystidia absent. Lamellar trama subregular to almost regular; hyphae 2.5–32 µm wide, thin-walled, hyaline to pale yellowish, faintly dextrinoid. Pileal trama subregular; hyphae 2–20 µm wide, slightly inflated, thin-walled, hyaline to pale yellowish. Pileipellis basically a cutis composed of hyphae that are covered entirely with minute excrescences and terminating in acanthocytes which overlap in such a way as to give an apparent subhymeniform appearance; hyphae 2.5–5.5 µm wide, thin-walled, hyaline; acanthocytes 18–54 × 10–41 µm, versiform: globose, subglobose, clavate, ovoid or sphaeropedunculate, thin-walled, hyaline; excrescences 0.5–2 × 0.5–1.5 µm, cylindrical or subconical; hypoderm composed of distinctly more inflated hyphae lacking excrescences. Pileus margin made up entirely of cells similar to cheilocystidia, 10–26 × 4.5–15.5 µm, thin-walled, hyaline. Spines of the universal veil made of cherocytes 25–90 × 2–31 µm, central and terminal ones mostly globose, clavate or fusiform, peripheral ones often cylindric, subcylindric or irregularly elongated, thick-walled (1–2 µm), with sparse excrescences, with 8–24 erect, pointed spine-like projections, 3–26 µm long. Stipitipellis a cutis with numerous caulocystidia; hyphae 2.5–13 µm wide, thin- to slightly thick-walled (0.25 µm), hyaline; caulocystidia 34.5–331.5+ × 6.5–15(–20) µm, long, scattered or in clusters, cylindrical, mostly with an obtuse apex, densely covered with excrescences all over. Both acanthocytes and cherocytes observed in the covering layers of the extreme base of the stipe; acanthocytes 11.5–71

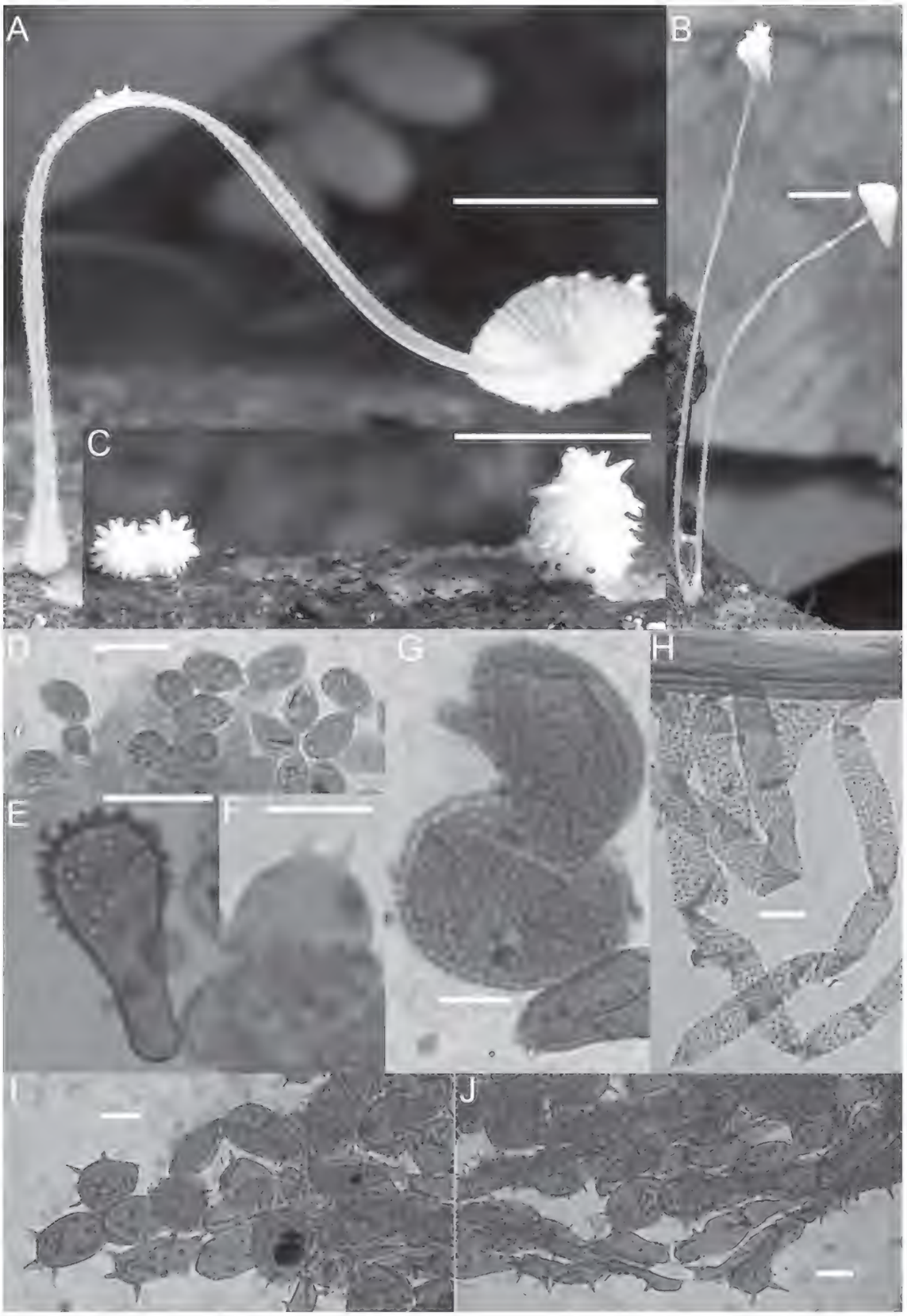

FIGURE 1, A–J: *Amparoina spinosissima*: A–B, basidiomata; C, primordia; D, spores; E, cheilocystidium; F, basidium; G, acanthocytes; H, stipitipellis and caulocystidia; I, cherocytes of terminal part of spine; J, cherocytes of basal part of spine. Scale bars: 5 mm for basidiomata and primordia and 10 µm for microscopic structures.

× 7.5–30.5 µm, subglobose to clavate, obpyriform or lageniform or nearly sphaeropedunculate, with evenly distributed excrescences 0.5–2 × 0.5–1.5 µm; cherocytes 23.5–33 × 12–27 µm, globose to subglobose or clavate, thick (1–2 µm)-walled, with excrescences all over, and with 5–12 pointed spine-like projections, up to 8 µm long. Clamp connections observed in all hyphae except at the base of caulocystidia and on pileipellis hyphae. Cherocytes of both the pileal surface and stipe base showed a tendency to germinate when mounted in water.

Habitat: On a decaying dicotyledonous twig, scattered or caespitose.

Collection examined—**INDIA, Kerala State, Calicut District**, Koyilandy, Poyilkaavu: 31 July 2009, D.M. Aravindakshan *DM314* [K(M)165810].

Discussion: The Indian collection shows all diagnostic characters of *A. spinosissima*, such as small, fragile, whitish basidiomata growing on dicotyledonous twigs, a universal veil composed of conical spines comprising thick-walled cherocytes, a pileipellis with detersile acanthocytes, a stipitipellis with very long and cylindrical caulocystidia with excrescences, cheilocystidia with excrescences, and non-discoid stipe base. However, some minor differences were noticed in the present collection compared to earlier descriptions. In their respective collections, Horak (1980) found all hyphae to be clampless and Desjardin (1995) found clamp connections only in the universal veil. On the contrary, we found clamp connections in most parts of the basidiomata of the Indian collections. While Desjardin found the cherocytes of the medullary region of the spines devoid of spine-like projections, all cherocytes had such projections in the present collections. Additionally, the maximum length of the cherocytes (90 µm), the maximum number of spine-like projections on the cherocytes (24), and the maximum length of the spine-like projections (26 µm) in the Indian collection were almost twice as much as what Desjardin (1995) has recorded. Also, in addition to the normal warty cheilocystidia, occasionally some totally smooth ones were seen. In view of these differences and the reported amyloid spores, Desjardin's collection may represent a different taxon.

As already mentioned, the reaction of the spores of *A. spinosissima* with Melzer's reagent has been a contentious issue and has a bearing on the autonomy of *Amparoina*. The spores of the Indian collection were found beyond any doubt to be inamyloid. This observation lends support for what Singer (1950, 1958, 1976, 1983) has recorded for the species and also for the autonomy of the genus. Another remarkable observation that we made on the Indian specimen is that the cherocytes from the veil tend to germinate when mounted in water. According to Singer (1983, 1986), the cherocytes of *Mycena* and *Amparoina* may be interpreted as chlamydospores.

This is the first record of *A. spinosissima* from continental Asia and it extends the known geographical distribution of this species beyond the Pacific Rim to

South Asia. Our findings support Singer's (1983) contention that *A. spinosissima* has a disjunct distribution and this may be indicative of its primitiveness.

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